REMARKS

A Petition to extend the time for responding to the outstanding Office Action, for three (3) months, under 37 C.F.R. §1.136(a), is enclosed with this paper.

Claims 1-44 are pending in this application.

Claims 23, 24, 26-34, 36-38 and 43 have been cancelled, without prejudice.

Claims 1, 18, 22, 24, 35, 39, 41, 42 and 44 have been amended to more particularly point out and distinctly claim the subject matter of Applicants invention. Claim 23 has been rewritten and is entered as new claim 45. No new matter is added by amendment to these claims or by entry of new claim 45.

Applicants respectfully reserve the right to pursue the subject matter of claims 24, 26-34, 36-38 and 43 in a future continuing application.

Applicants would like to take this opportunity to note that the continuing data for this application is as follows:

This application is a continuation of U.S. application serial no. 08/702,502, filed March 3, 1997, now abandoned, which is the §371 U.S. national phase prosecution of PCT international application serial no. PCT/US95/02633, filed March 3, 1995, which is a continuation-in-part of U.S. application serial no. 207,525, filed March 7, 1994, now abandoned. The specification was amended to recite this data in Applicants Amendment submitted October 24, 2000.

Rejection of Claims 24, 26-34, 36-38, and 43 Under 35 U.S.C. §112, first paragraph

Claims 24, 26-34, 36-38 and 43 remain rejected under §112, first paragraph, as discussed in the January 17, 2001 Office Action. Applicants respectfully disagree with the ongoing basis for this rejection. Regardless, in order to speed prosecution of the remaining pending claims, Applicants cancel claims 24, 26-34, 36-38 and 43. Cancellation of these claims is not to be considered an form of acquiescence on the part of Applicants regarding this portion of their invention. To this end, Applicants respectfully reserve the right to continue prosecution of this subject matter in a future continuing application or applications. For now, however, Applicants respectfully take the position that the rejection is overcome by cancellation of claims 24, 26-34, 36-38 and 43.

Rejection of Claims 1-44 under 35 U.S.C. §112, second paragraph

Claims 1-44 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants respectfully overcome this rejection by amending claims 1, 18, 22, 24, 35, 39, 41, 42 and 44, as well as canceling claim 23 and entering this subject matter as new claim 45.

More specifically, claim 1 is amended to clearly recite that placement of transcription termination sequences are dependent on the placement of promoter fragments throughout the polynucleotide vector. For example, placement of a transcription termination sequence after the second cistron would necessitate placement of a promoter fragment upstream of an optional third cistron to drive expression of the third gene. Therefore, Applicants respectfully take the position that amendment to claim 1 results in a clear recitation of the metes and bounds of this portion of Applicants invention.

Claim 18 is amended, as suggested by the Examiner, to more clearly recite that the polynucleotide vector will not replicated within the *in vivo* target cell.

Claim 22 is amended, as suggested by the Examiner, to correct any confusion as to reference to "first" and "second" genes within the polynucleotide vector.

Claims 24 and 35 are amended, as suggested by the Examiner, to contain appropriate Markush language. Claim 35 is further amended in incorporate appropriate transitional language between element e) and f) of the claim. Claim 35 is again amended to correct an editorial oversight regarding the phrase "an eukaryotic,", as noted by the Examiner regarding claims 39 and 41.

Claim 41, as suggested by the Examiner, is amended to correct the editorial oversights regarding the terms "an eukaryotic" and "an heterologous." Claim 41 has been further amended to more clearly distinguish between a first and second open reading frame within the claimed polynucleotide expression vector.

Claim 42, as suggested by the Examiner, is amended to more clearly recite the basic concept that cistrons encoding foreign antigens are located within the confines of the deliverable polynucleotide vector. It is the combination of multiple polynucleotide vectors into a single formulation which constitutes the basis for claim 42.

Claim 44 is amended to specifically recite polynucleotide vector limitations, instead of relying on the basis of Figure 2 for an interpretation of such limitations. More specifically, claim 44 is amended to incorporate the limitation of twice amended claim 1.

Claim 23 is cancelled and new claim 45 is added, which recites in Markush style claim language (without information disclosed elsewhere in the specification) preferred polynucleotide constructs utilized in practicing the present invention.

To this end, Applicants respectfully take the position that cancellation of claim 36 and 43, amendment to claims 1, 18, 22, 24, 35, 36, 39, 41, 42, and 44, as well as replacement of claim 23 with new claim 45, effectively overcomes the basis for the present §112, second paragraph rejection. Therefore, Applicants respectfully request that this rejection be withdrawn and all remaining claims be allowed. The Examiner is invited to contact the undersigned attorney if clarification is required on any aspect of this response, or if any of the claims are considered to require further amendment to be placed in condition for allowance after entry of this Amendment.

Respectfully submitted,

Date: July 16, 2001

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MARKED-UP VERSION OF APPLICATION AS AMENDED HEREIN

IN THE CLAIMS:

1(Twice Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;

b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and

d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by [another cistron lacking] a second cistron or third cistron, respectively, which lacks its own transcriptional promoter.

18(Twice Amended). A polynucleotide which [comprises contiguous nucleic acid sequences which] cannot replicate in eukaryotic cells *in vivo* and which comprises contiguous nucleic acid sequences [but which are] capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues *in vivo*, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

a) a spliced REV gene;

b) a spliced human immunodeficiency virus (HIV) immunogenic epitope; and,

c) optionally, a cytokine or a T-cell recognition element.

22(Twice Amended). A polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the first gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the first gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the first gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene encoding a REV gene product, wherein said polynucleotide is non-replicating in eukaryotic cells *in vivo*.

24(Twice Amended) A polynucleotide which is non-replicating in eukaryotic cells *in vivo* and induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue *in vivo*, wherein the polynucleotide comprises a gene encoding a gene product selected from the group consisting of HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

35(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

a) [an] a eukaryotic transcriptional promoter;

b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;

c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;

d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes[, the genes] being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,

f) a transcription-termination signal following the last open reading frames.

39(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

a) [an] a eukaryotic transcription initiation signal;

b) an HIV gene open reading frame (ORF) preceded by [an] a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;

d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and

e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

41(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

a) [an] a eukaryotic transcription initiation signal;

b) [an] a first HIV gene open reading frame (ORF) preceded by [an] a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the first HIV ORF;

d) [an] a second HIV gene open reading frame (ORF) preceded by [an] a heterologous leader sequence such that expression of the second HIV gene ORF does not depend on availability of the HIV REV gene product; and

e) a transcription termination signal 3' to the translation stop codon of the second HIV gene ORF.

42(Twice Amended). A composition comprising multiple [expression constructs] polynucleotide expression vectors of claim 1, each polynucleotide expression vector, [of which is] upon *in vivo* introduction into a mammalian cell, being non-replicating but being capable of inducing expression [in mammalian tissue] of more than a single cistron contained within the polynucleotide expression vector, the cistrons encoding antigens related to disease causing pathogens or tumors.

44(Amended). [A polynucleotide construct which is non-replicating in eukaryotic cells *in vivo*, having the elements shown in figure 2,] A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter; wherein each of the first, second and optionally third cistrons [shown in the figure] encode a combination of any two to three of the following:

1) tPA-gp120$_{MN}$;

2) gp160$_{IIIB}$/IRES/*REV*$_{IIIB}$;

3) gp160$_{IIIB}$;

4) *REV*$_{IIIB}$;

5) *tat*/*REV*/gp160;

6) REV/gp160;

7) gp160$_{MN}$;

8) gp160 from clinically relevant primary HIV isolates;

9) nef, using the gene from clinically relevant strains;

10) *gag*$_{IIIB}$;

11) tPA-gp120$_{IIIB}$;

12) gp160 with structural mutations including V3 loop substitutions from clinically relevant strains of HIV; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;

13) gp41 with provision of appropriate leader sequences, as in the tPA signal peptide leader sequence;

14) *gag*: similar to construct from #5 above, using the gene from clinically relevant strains;

15) *rev*: for gp160 and *gag* dicistronics;

16) B7 coding sequences;

17) GM-CSF sequences;

18) Interleukin sequences;

19) Tumor associated antigens;

20) Genes encoding antigens expressed by pathogens other than HIV, such as, but not limited to, influenza virus nucleoprotein, hemagglutinin, matrix, neuraminidase, and other antigenic proteins; herpes simplex virus genes; human papillomavirus genes; tuberculosis antigens; hepatitis A, B, or C virus antigens; and combinations of these and other antigens to form at least dicistronic constructs which may be combined with multiple other polycistronic constructs to provide a cocktail composition capable of raising immune responses against all of the represented pathogens or tumor antigens[;

wherein the segments A and B of figure 2 are internal ribosome entry sites or a combination of transcription termination sequences terminating the transcription of the upstream cistron and transcriptional promoter sequences, initiating the transcription of downstream cistron].

New claim 45 was entered as follows:

45(New). The polynucleotide construct selected from the group consisting of V1Jns-(tat/rev SD), V1Jns-gp160$_{IIIB}$/IRES/rev $_{IIIB}$ (SD), V1Jns-gag-prt$_{IIIB}$ (SD), V1Jns-gag-prt$_{IIIB}$, V1Jns-tPA, V1Jns-tPA-gp120$_{MN}$, V1J-SIV$_{MAC251}$p28 gag, V1J-SIV$_{MAC251}$nef, and V1Jns-tat/rev/env.